The Synthesis and Analgesic Activities of Some Spiro[indan-1,3′-pyrrolidine] Derivatives Designed as Rigid Analogs of Profadol

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Abstract □ Aromatic hydroxylated derivatives of spiro[indan-1,3′-pyrrolidine], designed as conformationally restricted analogs of profadol, were synthesized and pharmacologically evaluated in mice for analgesia and other central nervous system activities. None of the compounds synthesized were as potent as profadol in writhing and hot plate tests, but the 4-hydroxy derivative exhibited codeine-level analgesia in the tests.

Keyphrases □ Profadol—rigid analogs, spiro[indan-1,3′-pyrrolidine] derivatives, synthesis and analgesic activity □ Analgesic activity—evaluation of some spiro[indan-1,3′-pyrrolidine] derivatives, synthesis □ Spiro[indan-1,3′-pyrrolidine] derivatives—synthesis and evaluation for analgesic activity

As part of this laboratory's interest in the use of spiro-compounds as analgesic receptor probes (1–3), a series of spiro[tetralin-1,3′-pyrrolidine] (I) derivatives, designed as conformationally and rotationally restricted analogs of the analgesic drug, profadol (II), were synthesized recently and their analgesic activities determined. The present report describes the synthesis and analgesic activities of some structurally related spiro[indan-1,3′-pyrrolidine] (III) derivatives in which conformational flexibility is restricted more than in I.

EXPERIMENTAL[1]

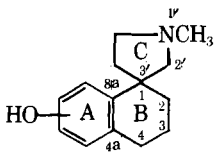
Chemistry—*Ethyl 4-Methoxy-indanylidenecyanoacetate* (*IV*b)—A mixture of 4-methoxy-1-indanone (16.2 g, 0.1 mole), ethyl cyanoacetate (12.43 g, 0.11 mole), ammonium acetate (15.4 g, 0.2 mole), and acetic acid (48 g, 0.8 mole) in benzene (100 ml) was heated under reflux with a Dean and Stark water trap for 24 hr. The cooled reaction mixture was washed with water (3 × 100 ml), the washings extracted with benzene (2 × 50 ml) and the combined benzene liquors dried (magnesium sulfate) and concentrated to give crude product. Recrystallization from ethanol gave an analytical sample (Table I); NMR (dimethyl sulfoxide-d_6): δ 1.38 (t, 3H, OCH_2CH_3), 2.98 (m, 2H, indan-3 protons), 3.52 (m, indan-2 protons), 3.86 (s, 3H, OCH_3), 4.32 (q, 2H, OCH_2CH_3), 7.00 (1H, m, indan-5 proton), 7.29 (1H, m, indan-6 proton), and 8.26 (1H, m, indan-7 proton) ppm; IR: 2215 (C≡N) and 1720 (C=O) cm^{-1}.

Compounds IV*a*, IV*c*, and IV*d* were prepared similarly from the appropriately substituted 1-indanone[2] (Table I). The IR and NMR spectra were consistent with their assigned structures.

4-Methoxy-1-cyano-1-cyanomethylindane (*IVf*)—A solution of potassium cyanide (16.2 g, 0.25 mole) in water (50 ml) was added to a stirred solution of IV*b* (24.5 g, 0.1 mole) in absolute ethanol (200 ml) and the contents stirred at 65° for 16 hr. The mixture was then evaporated to dryness *in vacuo*, the residue suspended in water (200 ml) and extracted with ether (3 × 200 ml). The ether extracts were washed with water (2 × 50 ml), dried (magnesium sulfate), and the solvent evaporated to give a brown oil. This oil was vacuum distilled to give IV*f*, a colorless oil that crystallized on standing. Recrystallization from ethanol gave an analytical sample (Table I); NMR (deuterochloroform): δ 2.40 (m, 2H, indan-2 protons), 2.86 (s, 2H, CH_2CN), 2.97 (m, 2H, indan-3 protons), 3.80 (s, 3H, OCH_3), 6.83 (m, 1H, indan-5 proton), 7.12 (m, 1H, indan-6 proton), and 7.33 (m, 1H, indan-7 proton) ppm; IR: 2255 and 2240 (both C≡N) cm^{-1}.

Compounds IV*e*, IV*g*, and IV*h* were prepared similarly from IV*a*, IV*c*, and IV*d*, respectively. IR and NMR spectra were consistent with the assigned structures.

4-Methoxy spiro[indan-1,3′-pyrrolidine-2′,5′-dione] (*IVj*)—A suspension of IV*f* (21.2 g, 0.1 mole) in acetic acid (50 ml) and 78% (v/v) sulfuric acid (20 ml) was heated in an oil bath at 125° for 1 hr. The acetic acid was removed by *in vacuo* distillation and the resulting mass was suspended in water (50 ml) and extracted with ethyl acetate (3 × 300 ml). The organic phase was washed with saturated sodium bicarbonate solution (2 × 200 ml) and water (100 ml) and then dried (magnesium sul-



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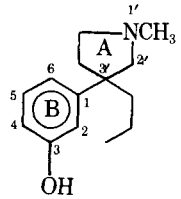

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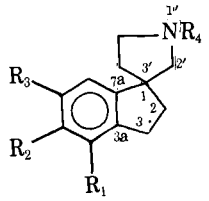
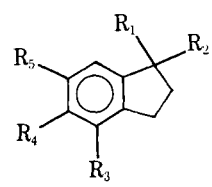


III

IIIa: $R_1 = R_2 = R_3 = R_4 = H$
IIIb: $R_1 = R_2 = R_3 = H$; $R_4 = CH_3$
IIIc: $R_1 = OCH_3$; $R_2 = R_3 = R_4 = H$
IIId: $R_1 = OCH_3$; $R_2 = R_3 = H$; $R_4 = CH_3$
IIIe: $R_1 = R_3 = R_4 = H$; $R_2 = OCH_3$
IIIf: $R_1 = R_3 = H$; $R_2 = OCH_3$; $R_4 = CH_3$
IIIg: $R_1 = R_2 = R_4 = H$; $R_3 = OCH_3$
IIIh: $R_1 = R_2 = H$; $R_3 = OCH_3$; $R_4 = CH_3$
IIIi: $R_1 = OH$; $R_2 = R_3 = R_4 = H$
IIIj: $R_1 = OH$; $R_2 = R_3 = H$; $R_4 = CH_3$
IIIk: $R_1 = R_3 = R_4 = H$; $R_2 = OH$
IIIl: $R_1 = R_3 = H$; $R_2 = OH$; $R_4 = CH_3$
IIIm: $R_1 = R_2 = R_4 = H$; $R_3 = OH$
IIIn: $R_1 = R_2 = H$; $R_3 = OH$; $R_4 = CH_3$



IV*a*–IV*l*
(Table I)

[1] Melting points were determined on a Reichert hot stage microscope and are uncorrected. IR spectra were determined as Nujol mulls on a Perkin-Elmer 237 spectrophotometer, and NMR spectra were determined on a Perkin-Elmer R12B instrument using tetramethylsilane as the internal standard.

[2] 1-Indanone and 5-methoxy-1-indanone were purchased from Aldrich Chemical Corp., 4-methoxy-1-indanone was prepared by the method of London and Razdan (4), and 6-methoxy-1-indanone was prepared by the method of House and Hudson (5).

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Table I—Physical and Analytical Data for 1,1-Disubstituted Indane Derivatives

Compound	R_1	R_2	R_3	R_4	R_5	Yield, %	Melting Point	Formula	Analysis, % Calc.	Found
IV*a*	$=C(CN)CO_2C_2H_5$		H	H	H	61.7	103–104°	$C_{14}H_{13}NO_2$	C 74.0	74.2
									H 5.7	5.5
									N 6.2	6.1
IV*b*	$=C(CN)CO_2C_2H_5$		$-OCH_3$	H	H	33.1	175–178°	$C_{15}H_{15}NO_3$	C 70.0	70.0
									H 5.9	5.9
									N 5.4	5.3
IV*c*	$=C(CN)CO_2C_2H_5$		H	$-OCH_3$	H	50.8	165–167° (subl.)	$C_{15}H_{15}NO_3$	C —	70.1
									H —	6.0
									N —	5.3
IV*d*	$=C(CN)CO_2C_2H_5$		H	H	$-OCH_3$	20.4	137–139°	$C_{15}H_{15}NO_3$	C —	70.1
									H —	6.0
									N —	5.3
IV*e*	CN	CH_2CN	H	H	H	83.5	54–56°	$C_{12}H_{10}N_2$	C 79.1	79.4
									H 5.5	5.5
									N 15.4	15.4
IV*f*	CN	CH_2CN	$-OCH_3$	H	H	84.7	71.5–73°	$C_{13}H_{12}N_2O$	C 73.6	74.0
									H 5.7	5.8
									N 13.2	13.2
IV*g*	CN	CH_2CN	H	OCH_3	H	57.7	52–55°	$C_{13}H_{12}N_2O$	C —	73.4
									H —	5.8
									N —	12.8
IV*h*	CN	CH_2CN	H	H	OCH_3	50.8	86–88°	$C_{13}H_{12}N_2O$	C —	74.1
									H —	5.9
									N —	13.0
IV*i*	$-CONHCOCH_2-$		H	H	H	69.7	151–153°	$C_{12}H_{11}NO_2$	C 71.6	71.7
									H 5.5	5.55
									N 7.0	6.8
IV*j*	$-CONHCOCH_2-$		OCH_3	H	H	61.6	187–189°	$C_{13}H_{13}NO_3$	C 67.5	67.4
									H 5.7	5.7
									N 6.1	6.0
IV*k*	$-CONHCOCH_2-$		H	OCH_3	H	66.7	174–176°	$C_{13}H_{13}NO_3$	C —	67.4
									H —	5.4
									N —	6.1
IV*l*	$-CONHCOCH_2-$		H	H	OCH_3	73.0	154–156°	$C_{13}H_{13}NO_3$	C —	67.6
									H —	5.7
									N —	5.8

fate). Evaporation of the solvent gave the crude product which was recrystallized from absolute ethanol to give an analytical sample (Table I); NMR (dimethyl sulfoxide-d_6): δ 1.87–2.80 (m, 2H, indan-2 protons), 2.89–3.26 (m, 2H, indan-3 protons), 2.03 (s, 2H, pyrrolidine-4′ protons), 3.79 (s, 3H, OCH_3), 6.68 (m, 1H, indan-5 proton), 6.74 (m, 1H, indan-7 proton), 7.21 (m, 1H, indan-6 proton), and 8.50 (broad s, 1H, exchangeable with deuterium oxide, −NH) ppm; IR: 3190, 3075, 1780, 1725, and 1695 cm^{-1}.

Compounds IV*i*, IV*k*, and IV*l* were prepared similarly from IV*e*, IV*g*, and IV*h*, respectively; IR and NMR spectra were consistent with the assigned structures.

4-Methoxy spiro[indan-1,3′-pyrrolidine] (*III*c)—Finely divided IV*j* (23.1 g, 0.1 mole) was added slowly to a stirred suspension of aluminum lithium hydride (11.25 g, 0.3 mole) in dry tetrahydrofuran and the mixture refluxed for 24 hr. The excess aluminum lithium hydride was decomposed by careful addition of water, anhydrous magnesium sulfate was added, and the suspension was filtered. The filtrate was evaporated *in vacuo* to give a pale yellow oil which was distilled *in vacuo* to give III*c;* NMR (deuterochloroform): δ 1.78–2.22 (m, 4H, indan C-2 and C-4′ protons), 2.05 (s, 1H, exchangeable with deuterium oxide, NH), 2.69–2.97 (m, 2H, C-3 protons), 2.90 (s, 2H, C-2′ protons), 2.07–3.29 (m, 2H, C-5′ protons), 3.76 (s, 3H, OCH_3), 6.66 (m, 1H, C-5 proton), 6.80 (m, 1H, C-7 proton), and 7.17 (m, 1H, C-6 proton) ppm. The base was converted to the fumarate salt which was recrystallized from ethanol–ether to give an analytical sample (Table II).

Compounds III*a*, III*c*, and III*g* were prepared in a similar manner from IV*i*, IV*k*, and IV*l*, respectively and converted to the hydrochloride or fumarate salts (Table II). IR and NMR spectra were consistent with the assigned structures.

*4-Methoxy-*N*-methyl spiro[indan-1,3′-pyrrolidine]* (*III*d)—A solution of III*c* (2.0 g, 0.01 mole) in formic acid (2.3 g, 0.05 mole) and 40% formaldehyde solution (2 ml, 0.02 mole) was heated with stirring at 95° for 24 hr. The reaction mixture was diluted with 5% hydrochloric acid (5 ml) and extracted with ether (2 × 10 ml). The aqueous phase was made basic with concentrated ammonia solution, and extracted with ether (3 × 20 ml). The combined layers were washed with water (10 ml), dried (magnesium sulfate), and the solvent evaporated. The resulting oil was distilled *in vacuo* to give pure III*d;* NMR (deuterochloroform): δ 1.88–2.30 (m, 4H, C-2 and C-4′ protons), 2.35 (s, 3H, N—CH_3), 2.50–2.98 (m, 4H, C-3 and C-5′ protons), 2.64 (s, 2H, C-2′ protons), 3.77 (s, 3H, OCH_3), 6.64 (m, 1H, C-5 proton), 6.90 (m, 1H, C-7 proton), and 7.20 (m, 1H, C-6 proton) ppm. The base was converted to the fumarate salt which was recrystallized from ethanol–ether to give an analytical sample (Table II).

Compounds III*b*, III*f*, and III*h* were prepared in a similar manner from III*a*, III*e*, and III*g*, respectively, and converted to either their hydrochloride or fumarate salts (Table II). Their IR and NMR spectra were consistent with the assigned structures.

4-Hydroxy spiro[indan-1,3′-pyrrolidine]hydrobromide (*III*i)—A solution of III*c* (2.0 g, 0.01 mole) in 48% aqueous hydrobromic acid (25 ml) was refluxed under nitrogen for 1.5 hr. The yellow-brown solution was chilled to give buff crystals of III*i* which were recrystallized from ethanol–ether, giving an analytical sample (Table II); NMR (deuterium oxide): δ 1.80–2.20 (m, 4H, C-2 and C-4′ protons), 2.55–2.90 (m, 2H, C-3 protons), 3.21 (s, 2H, C-2′ protons), 3.24–3.68 (m, 2H, C-5′ protons), 6.51 (m, 1H,C-5 proton), 6.69 (m, 1H, C-7 proton), and 7.02 (m, 1H, C-6 proton) ppm; IR: 3210 (–OH), 2720 (NH) cm^{-1}.

Compounds III*j*–III*n* were prepared similarly from the appropriately substituted spiro[indan-1,3′-pyrrolidine]. Their IR and NMR spectra were all consistent with the assigned structures (Table II).

Pharmacology—Analgesia was determined by the acetic acid writhing test (6) in groups of six mice. Each group was dosed orally with either vehicle[3] or the compound under test and injected intraperitoneally 30 min later with dilute acetic acid (0.4 ml, 0.25%). Compounds showing analgesia at 50 mg/kg in that test were also tested for analgesia by the hot-plate method (7) (Table II).

Locomotor activity was determined using a battery of photobeam cages (30 × 12 × 9-cm) traversed by a beam of light. Interruptions of the beam were counted automatically. Groups of 10 mice were dosed and placed singly in the cages. After 45 min the total number of interruptions recorded and the percentage of inhibition or increase in movement relative to controls were determined.

Reversal of reserpine-induced hypothermia was assessed in groups of

[3] The testing vehicle used was "Dispersol," ICI Ltd., Pharmaceuticals Division, Macclesfield, UK, which consists of Lissapol NX (Ex ICI Dyestuffs Division, nonylphenol–ethylene oxide concentrate) (0.1%), "Lissapol" C** (sodium salt of sulfated cetyl/oleyl alcohol mixture) (0.1%), "Dispersol" OG** (polyglyceryl ricinoleate) (0.1%), and water (99.7%).

Table II—Physical, Chemical, and Pharmacological Data for Spiro[indan-1,3′-pyrrolidine] Derivatives

Compound	Yield, %	Melting Point	Formula	Analysis, % Calc.	Analysis, % Found	Writhing Test (Mice) ED_{50}, mg/kg po[a]
III*a* HCl	79.3	122–125°	$C_{12}H_{15}N$·HCl	C 68.7 H 7.7 N 6.7	68.7 7.6 6.6	>50
III*b* HCl	86.4	219–221°	$C_{13}H_{17}N$·HCl	C 69.8 H 8.1 N 6.3	69.7 8.2 6.4	13.9 (11.1–16.3)[b,c]
III*c* fumarate	98.9	188–195°	$C_{13}H_{17}NO{\cdot}C_4H_4O_4$	C 63.9 H 6.6 N 4.4	64.1 6.6 4.5	31.3 (23.2–39.6)[d]
III*d* fumarate	89.2	150–155°	$C_{14}H_{19}NO{\cdot}C_4H_4O_4$	C 64.9 H 6.9 N 4.2	65.1 7.1 4.2	*NA*
III*e* fumarate	89.8	168–175°	$C_{13}H_{17}NO{\cdot}C_4H_4O_4$	C 63.9 H 6.6 N 4.4	64.0 6.6 4.3	*NA*
III*f* fumarate	81.9	146–150°	$C_{14}H_{19}NO{\cdot}C_4H_4O_4$	C 64.9 H 6.9 N 4.2	64.7 6.7 3.8	*NA*
III*g* fumarate	92.4	124–130°	$C_{13}H_{17}NO{\cdot}C_4H_4O_4$	C 63.9 H 6.6 N 4.4	64.1 6.6 4.25	*NA*[e]
III*h* fumarate	93.5	140–147°	$C_{14}H_{19}NO{\cdot}C_4H_4O_4$	C 64.9 H 6.9 N 4.2	64.7 6.7 4.0	30.1 (19.1–38.2)[d]
III*i* HBr	75.2	181–187°	$C_{12}H_{15}NO$·HBr	C 53.4 H 6.0 N 5.2	53.3 6.1 5.3	12.5 (8.9–15.1)[f]
III*j* HBr	75.2	231–237°	$C_{13}H_{17}NO$·HBr	C 54.9 H 6.4 N 4.9	55.1 6.4 4.7	*NA*
III*k* HBr	92.8	222–229°	$C_{12}H_{15}NO$·HBr	C 53.4 H 6.0 N 5.2	53.6 6.1 5.2	>50
III*l* HBr	57.3	272–278°	$C_{13}H_{17}NO$·HBr	C 54.9 H 6.4 N 4.9	54.9 6.3 4.9	>50[g]
III*m* HBr	87.9	263–267°	$C_{12}H_{15}NO$·HBr	C 53.4 H 6.0 N 5.2	53.2 5.9 5.0	*NA*
III*n* HBr	94.3	182–187°	$C_{13}H_{17}NO$·HBr	C 54.9 H 6.4 N 4.9	54.8 6.3 4.9	*NA*
Morphine						1.4 (0.7–3.6)[h]

[a] Confidence limits in parentheses. [b] Effective at 30 mg/kg in reversing pentylenetetrazol-induced convulsions in mice. [c] Failed to exhibit a normal dose-response curve in the hot-plate test. [d] Not active at 50 mg/kg po in hot-plate test. [e] Exhibited central stimulant activity at 30 mg/kg in mice. [f] ED_{50} = 16.8 (11.6–25.3) mg/kg po in hot-plate test. [g] Caused a 90% reduction in motility at 30 mg/kg in mice. [h] ED_{50} = 3.8 (2.2–6.5) mg/kg po in hot-plate test. *NA* Not active at 100 mg/kg.

six mice as described previously (8) and based on the method of Askew (9).

Anticonvulsant activity was measured in groups of six mice subjected to the application of maximal electroshock (20 mamp for 0.22 sec *via* aural clip electrodes) or administration of the chemical convulsant, pentylenetetrazol (160 mg/kg subcutaneously). In each case, tonic extensor spasm was used as the end-point.

Impairment of motor coordination was assessed by measuring the ability of groups of six mice to balance on a stationary 1.3-cm diameter metal rod. Individual animals were placed on the rod and observed throughout the subsequent 20 sec. The time each mouse remained upon the rod was recorded and converted to a score. If the mouse balanced for the entire 20 sec, a score of 0 was given; if the mouse fell off after T sec, a score of $20 - T$ was given. Mice that fell off the rod on their first attempt were replaced for three more trials, providing a maximum possible score of 80. The mean score per mouse was determined and compared by standard statistical procedures with corresponding values obtained for groups treated with vehicle alone.

RESULTS

Chemistry—The syntheses of compounds IV*a*–IV*l* and III*a*–III*h* were carried out using procedures similar to those described previously for the preparation of the spiro[tetralin-1,3′-pyrrolidine] derivatives (3), but utilizing the appropriately substituted 1-indanone in place of the corresponding 1-tetralone. The appropriate 1-tetralone was condensed with ethylcyanoacetate to give the 1-indanylidenecyanoacetate derivative (IV*a*–IV*d*), which was reacted with potassium cyanide to give the corresponding 1-cyano-1-(cyanomethyl)indane (IV*e*–IV*h*). Treatment of compounds IV*e*–IV*h* with an acetic acid–sulfuric acid mixture gave the corresponding spiro[indan-1,3′-pyrrolidine-2′,5′-dione] derivatives (IV*i*–IV*l*). These compounds were then reduced with aluminum lithium hydride in tetrahydrofuran to give the appropriately substituted spiro[indan-1,3′-pyrrolidine] (III). *O*-Demethylation of compounds III*c*–III*h* was carried out under nitrogen in refluxing 48% hydrobromic acid containing 1% phosphoric acid. *N*-Methylation of compounds III*a*, III*c*, III*e*, and III*g* was accomplished in formic acid–formaldehyde solution (Tables I and II).

Pharmacology—Compounds III*b* and III*i* were the most active compounds in the series, exhibiting codeine-level analgesia, whereas compound III*j* showed no analgesic activity. None of the other hydroxylated derivative (III*k*–III*n*) showed any significant analgesic activity, although compound III*l* caused marked reduction in motility in mice at a 30-mg/kg dose, whereas compound III*g* exhibited central stimulant properties at 30 mg/kg in mice. Compound III*b* protected mice against pentylenetetrazol induced convulsions at 30 mg/kg. Compounds III*a*–III*n* were inactive in the maximal electroshock test, in reserpine antagonism tests, and did not produce any impairment of coordination in the stationary rod test.

DISCUSSION

An important feature of profadol (II) structure is the rotational freedom present in the molecule which allows a number of rotameric forms as the aromatic ring is rotated about the C1–C3′ bond. Thus, the weak analgesic activity exhibited by compounds of structure I possibly results from these compounds adopting conformations that are noncomplimentary with the receptor. Spiro[indan-1,3′-pyrrolidine] (III) derivatives may be regarded as profadol analogs, which are more conformationally rigid than the spiro system (I), since aromatic ring rotation is severely

restricted in III as a result of the reduction of ring B to a five-membered ring. Thus, compounds III*j*, III*l*, and III*n* represent interesting rigid analogs of II. Furthermore, molecular model examination of III*j* and morphine shows the phenolic and tertiary amino groups, and the aromatic ring in both compounds to be almost stereosuperimposable. In addition, it was of interest to find a patent describing some 3-hydroxyspiro[indan-1,3'-pyrrolidine] derivatives as orally active analgesics (10). Profadol is thought to interact with the analgesic receptor in the half-chair conformation (11).

Although the spiro systems I and III represent structural analogs in which the profadol molecule is fixed in a conformation related to the morphine molecule, these compounds exhibit only weak or insignificant analgesic properties. Thus, other structural features appear to be important in profadol-receptor interaction. The inability of these spiro analogs to enhance or maintain the analgesic properties of profadol may be related to a decreased fit of these compounds at the receptor due to the rigid orientation of the B ring in these compounds, which represents a fixed, and perhaps undesirable, conformation of the 3-propyl group in II.

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Quantitation of Hydroxyprogesterone Caproate, Medroxyprogesterone Acetate, and Progesterone by Reversed-Phase High-Pressure Liquid Chromatography

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Abstract □ A high-pressure liquid chromatographic method for the quantitation of hydroxyprogesterone caproate, medroxyprogesterone acetate, and progesterone in pharmaceutical dosage forms was developed. The method gave accurate, precise, and reproducible results. The excipients present in the dosage forms did not interfere with the assay procedure except benzyl benzoate in progesterone injection. The percent relative standard deviations based on six injections were 1.6, 2.5, and 2.7% for hydroxyprogesterone caproate, medroxyprogesterone acetate, and progesterone, respectively. The stability of progesterone in ethanol–propylene glycol–water (10:50:40) was studied. The loss in potency of progesterone, even after 487 days of storage at 50°, was <10%.

Keyphrases □ Hydroxyprogesterone caproate—quantitation by reversed-phase high-pressure liquid chromatography □ Medroxyprogesterone acetate—quantitation by reversed-phase high-pressure liquid chromatography □ Progesterone—quantitation by reversed-phase high-pressure liquid chromatography □ High-pressure liquid chromatography—quantitation of hydroxyprogesterone caproate, medroxyprogesterone acetate, and progesterone

The pharmaceutical dosage forms of hydroxyprogesterone caproate (I), medroxyprogesterone acetate (II), and progesterone (III) are used extensively. The USP method (1) for the quantitative determination of I in dosage forms is based on a reaction with isoniazid. The color produced is measured spectrophotometrically.

For dosage forms of II, the USP method (2) requires normal phase high-pressure liquid chromatography (HPLC) with a porous silica column. The quantitation of III requires reversed-phase chromatography (3). The 1-m column for this procedure has to be packed with octadecylsilane chemically bonded to silica gel. The USP method (3) appears to be a modification of a procedure recommended previously (4).

This investigation attempted to develop a reversed-phase HPLC method (with prepacked column) suitable for the quantitation of I, II, and III in pharmaceutical dosage forms. The stability of III in some aqueous systems was also determined.

EXPERIMENTAL

Reagents and Chemicals—All reagents and chemicals were ACS, USP, or NF grade and were used as received. 17-Hydroxyprogesterone[1], hydroxyprogesterone caproate[1], medroxyprogesterone acetate[2], and progesterone[3] were used without further purification.

Apparatus—The chromatograph[4] was connected to a multiple-wavelength detector[5], a recorder[6], and an integrator[7]. All pH values were determined using a pH meter[8].

Column—The column[9] (30 cm × 4-mm i.d.) was of a semipolar material consisting of a monomolecular layer of cyanopropylsilane permanently bonded by silicone–carbon bonds.

Chromatographic Conditions—The mobile phase contained 30%

[1] E. R. Squibb & Sons, Princeton, N.J.
[2] The Upjohn Co., Kalamazoo Mich.
[3] Aldrich Chemical Co., Milwaukee, Wis.
[4] Model ALC 202 equipped with a U6K universal injector, Waters Associates, Milford, Mass.
[5] Spectroflow monitor SF770, Schoeffel Instruments Corp., Westwood, N.J.
[6] Omniscribe 5213-12, Houston Instruments, Austin, Tex.
[7] Autolab minigrator, Spectra-Physics, Santa Clara, Calif.
[8] Model 4500 digital pH meter, Beckman Instruments, Irvine, Calif.
[9] μBondapak/CN, Waters Associates, Milford, Mass.

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